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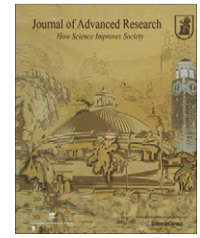

ORIGINAL ARTICLE

Impact of different biochemical markers in serum of patients with benign and malignant liver diseases

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Abstract The only hope for effective treatment of liver cancer lies in early detection or screening for populations who are at high risk for developing liver cancer. This study was designed to study the levels of a collection of biochemical markers in the sera of patients suffering from hepatocellular carcinoma (HCC) and its predisposing diseases. The ultimate aim is to investigate their diagnostic impact in the early detection of HCC and discriminate from benign liver diseases. The study was carried out on 217 individuals divided into the following groups: Group 1: Normal controls, Group 2: Schistosomal patients (Schist), Group 3: Hepatitis B patients (HBV), Group 4: Hepatitis C patients (HCV), Group 5: Cirrhotic patients (Cirr), and Group 6: Hepatocellular carcinoma patients (HCC). The last group was further subdivided into the following subgroups: a – HCC alone; b – HCC on top of schistosomiasis; c – HCC on top of HBV; d – Hepato-cellular carcinoma on top of HCV; e – HCC on top of cirrhosis. Their sera were subjected to a quantitative determination of the tumour necrosis factor-alpha (TNF-α), epidermal growth factor and its receptor (EGF and EGFR), glutathione-S-transferase alpha (GST-α), iron, ferritin, transferrin, alpha-1-antitrypsin (α1AT) and alpha-fetoprotein (αFP). The results of this study indicate that it is advisable to deter-

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mine a panel of markers composed of αFP, TNF-α and GST-α to confirm diagnosis of HCC and distinguish it from other benign liver diseases.

Introduction

In Egypt, digestive-system malignancies rank as the forth most common cancer, following the lympho-hematopoietic system, the breast and the urinary bladder. They contribute to 13.5% of total malignancies with a slight male predominance of 54.6% and a high adult-age predominance of 96.6%. Liver cancer forms more than 11.8% of these malignancies, about 70.5% of which are HCC [1]. HCC is multifactorial in origin and a number of causal associations have been identified. These may be divided into major and minor risk factors. The major risk factors include chronic HBV and HCV infection, which are represented in 70–95% of HCC patients [2], chronic necro-inflammatory hepatic disease, commonly in the form of cirrhosis, which is represented in 60–80% of patients [3]. HCC occurs in cirrhotic patients associated with HBV infection. However, 10–25% of cases developed in the absence of cirrhosis. This is due to the direct oncogenic effect of HBV as in the HBV-DNA genome, which integrates with hepatocellular chromosomes [4]. In contrast to HBV, HCV cannot integrate into the host genome. It exerts its effect, most probably, through production of cirrhosis with severe liver damage [5]. Many recent studies have shown that HCV has a direct oncogenic action through its core component [6]. Aflatoxin B1 is the most potent chemical known to cause HCC. It is a product of a mould called *Aspergillus flavus,* which grows in oily seeds that have been stored in a hot and humid environment, such as peanuts, soybeans, corn and wheat. It is thought to cause cancer via mutations in the p53 gene, by interfering with its tumour suppressing functions [7].

Minor risk factors include oral steroidal contraceptives and androgens that are associated with the development of hepatic adenomas, which have the potential to become malignant. It also includes cigarette smoking, membranous obstruction of the inferior vena cava, and a variety of mostly rare inherited metabolic diseases, particularly hereditary haemochromatosis [8].

Both HCV and HBV infection are the most common risk factors of HCC among Egyptian patients. About 10–20% of the general Egyptian population is infected with HCV [9]. Approximately 90% of Egyptian HCV isolates belong to a single subtype, 4a, which responds less successfully to interferon therapy than other subtypes [10]. Farmers who are exposed to chemicals during their work, such as insecticides, herbicides, pesticides and fertilizers are very likely to develop hepatoma. Schistosomiasis also increased the severity of HBV infection and elevated the risk of HCC over that associated with the HBV infection alone. Other factors associated with an increased risk of HCC in Egypt include cigarette smoking and occupational exposure to chemicals [11–13].

Liver cancer grows silently and does not cause symptoms until the disease is advanced, at which time there is little chance for recovery, and life expectancy is usually in the range of a few months. Therefore, the only hope of effective treatment lies in early detection with screening of high-risk populations. Two tests are commonly used to screen for liver cancer, namely an ultrasound examination of the liver and analysis of serum level of αFP [14]. Both tests have advantages and disadvantages. The αFP is easier to do and less expensive. However, it is not 100% specific and sensitive, since minor elevations are common in patients with chronic liver disease, pregnancy and germ cell tumours. Titers also rise with flares of active hepatitis, and may be persistently elevated in patients with cirrhosis [15]. Ultrasound is better, but more expensive, very operator dependent and less reliable in the presence of cirrhosis, missing a significant number of cancers. New screening of serum markers is under evaluation. None of them have yet been conclusively diagnostic.

This study was designed to determine the levels of several tumour markers in sera of patients suffering from HCC and other benign predisposing diseases, namely schistosomiasis, chronic active hepatitis B and C, as well as cirrhosis. The ultimate goal of the study is to find a panel of markers that would improve the early detection of HCC and screen for those who are at high risk for the disease, as well as distinguishing HCC from other benign liver lesions.

Subjects and methods

This work is a prospective study that lasted 18 months and started in December 2006. Two hundred and seventeen individuals were included in this study. They were selected from the out-patient clinic of the Department of Internal Medicine, National Liver Institute, Menoufia University, and the Department of Medical Oncology, National Cancer Institute, Cairo University. Patients were informed via documented consent. The ethical committee (IRD) of the NCI, Cairo University, approved the study in November 2006. This committee follows the Helsinki ethical rules.

Individuals under investigation were divided into the following groups:

Group 1: Normal control group, including 17 apparently healthy individuals. They were healthy volunteers including the working team, their relatives, friends and colleagues.
Group 2: Schistosomal group, including 40 patients suffering of *Schistosoma mansoni* infestation.
Group 3: HBV group, including 40 patients infected with HBV.
Group 4: HCV group, including 40 patients infected with HCV.
Group 5: Cirrhotic group, including 40 patients suffering from liver cirrhosis without viral infection.
Group 6: HCC group, including 40 patients.

All patients were subjected to clinical and radiological examination to confirm their diagnosis. Sera were collected from all groups and subjected to quantitative determination of the following biochemical parameters:

1. TNF-α was determined by ELISA technique, using a kit provided by Immunogenetics Company, Belgium.

2. EGF was determined by ELISA technique, using a Quantikine Immunoassay kit, USA.
3. EGFR was determined by ELISA technique, using a Calbiochem Immunoassay kit, USA.
4. GST-α was determined by ELISA technique, using a Hep kit from Brotrin International, Ireland.
5. Iron was determined by chemiluminescence, using a Sentinel kit, Italy.
6. Ferritin was determined by ELISA technique, using a Quorum ElA kit, Canada.
7. Transferrin was determined by ELISA technique, using the SPQTM antibody reagent SET2 for transferring, from INCSTAR Corporation, USA.
8. α1AT was determined by chemiluminescence, using the SPQTM antibody reagent SET2 for α1AT, from INCSTAR Corporation, USA.
9. α FP was determined by ELISA technique, using a Quorum Ela Kit, Canada.

Statistical methods

Sample size was estimated to include 15 cases for each group at an alpha error of 0.05 and a power of the study of 95%. This depends on the difference between cirrhotic and HCC patients in the level of TNF-α. We included more cases in each disease group, owing to the multiplicity of markers as well as the large number of groups. Data were analyzed using SPSS version 15. Numerical data were expressed as median and range. For quantitative data, comparison between the six groups was done using a Kruskal–Wallis test followed by a Schefe test on the ranks of different variables for pair-wise comparison of HCC against other groups. An ROC curve was used to deduce the most appropriate cut-off levels of all markers for diagnosis of HCC. A p-value < 0.05 was considered significant [16].

Results

Table 1 shows the number, gender and clinical-pathological features of individuals from all groups under investigation.

Table 2 illustrates the changes in different parameters to the median levels as well as their range in the different groups studied. Significant elevations were observed in the levels of TNFα, GST-α and αFP in the disease groups, giving a tremendous increase in the HCC group. The levels of EGF and EGFR were significantly lower in sera of all disease groups as compared with the control group. Both markers were significantly higher in HCC patients compared with the other disease groups. Serum iron concentration in patients with HBV, HCV and cirrhosis was significantly higher than the control, Schist and HCC groups. Cases with hepatitis had significantly higher ferritin as compared with other groups. Transferrin levels were significantly lower in the HCC group compared with other disease groups. Serum levels of αIAT were significantly higher in the HCC group relative to the other disease groups.

Tables 3–5 show the AUC, cut-off values, sensitivities and specificities of the different parameters investigated. The TNFα, EGFR, GST-α and αFP gave the best sensitivities and specificities for the control group versus HCC. However, the best sensitivities and specificities for the benign liver diseases taken collectively versus the HCC group was achieved by TNFα, GST-α and αFP (Table 3). The TNFα, EGF and EGFR gave the best sensitivities and specificities for HCC versus the HCV group and the TNFα, EGF,EGFR, GST-α and αFP were the best for the HCC versus HBV group (Table 4). The best sensitivities and specificities for HCC versus the Schist group and HCC versus Cirr were obtained by TNFα, EGFR, GST-α, EGF and αFP (Table 5).

Table 1 Clinico-pathological features of individual in different groups under investigation.

Parameter	Control	Schist*	HCV	HBV	Cirr*	HCC
Number	17	17	40	40	40	40
Male	12	34	33	34	36	36
Female	5	6	1	6	4	4
Age	39.7 ± 6.4 (30–52)	44 ± 7 (30–55)	44 ± 9 (25–60)	42 ± 7.2 (30–55)	48.9 ± 5.4 (40–60)	53.4 ± 12.8 (28–80)
Alk phase	36.8 ± 8.3 (22–52)	32.5 ± 8.1 (15–53)	62.7 ± 28.9 (20–129)	90 ± 19.2 (24–130)	63 ± 42.1 (70–152)	198.3 ± 122.3 (68–64.4)
sGOT	15.4 ± 5.8 (8–29)	25 ± 8.4 (18–54)	57 ± 17.6 (25–83)	34.4 ± 13.1 (13–63)	90.1 ± 24.1 (118–190)	79.5 ± 101.3 (8–461)
sGPT	14.3 ± 4.3 (13–25)	35.5 ± 13.1 (15–71)	44 ± 19.6 (8–77)	104.6 ± 19.4 (60–160)	30.1 ± 18.2 (1.8–87)	47.9 ± 38.8 (4–177)
Tot. bilirubin	0.5.3 ± 0.2 (0.2–0.8)	0.7 ± 0.2 (0–1.3)	0.9 ± 0.7 (2.2–2.9)	4.9 ± 1.9 (2.2–10.5)	1.3 ± 1.8 (0.1–7.5)	2.2 ± 1.3 (0.1–6.0)
HCV +ve cases	0	0	40	0	0	13
HBV +ve cases	0	0	0	40	0	6
Hematemesis +ve cases	0	0	12	0	0	1
Ascitis +ve cases	0	0	9	4	8	29
Grade I						9
Grade II						26
Grade III						5

Data are expressed as mean ± SD and number of cases.
Data between parentheses represents the range.
* Schist and Cirr are referred to Schistosomal and Cirrhotic group, respectively.

Table 2 The levels of different markers in sera of different groups under investigation.

Groups markers	Control (n = 17)	Schist (n = 40)	HBV (n = 40)	HCV (n = 40)	Cirr (n = 40)	HCC (n = 40)	p-Value*
TNF-α (pg/ml)	19.4 (18.4–20.8)	32.0 (22.0–43.5)	65.5 (40.0–502.0)	119.5 (60.0–150.0)	171.0 (130.0–230.0)	301.0 (220.0–350.0)	< 0.001
EGF (pg/ml)	106.0 (98.0–112.0)	27.8 (20.0–44.0)	14.0 (8.0–20.0)	40.0 (33.0–60.0)	21.3 (4.5–206.0)	89.0 (8.9–120.0)	< 0.001
EGFR (fm/ml)	444.0 (410.0–494.0)	162.5 (16.5–180.0)	187.5 (152.0–225.0)	230.0 (190.0–260.0)	262.8 (224.0–280.0)	325.0 (275.0–340.0)	< 0.001
GST-α (μg/l)	4.8 (3.4–6.0)	14.3 (10.5–20.0)	19.5 (9.0–80.0)	42.6 (28.0–78.0)	30.0 (20.0–45.5)	77.0 (31.0–191.0)	< 0.001
Iron (μg/ml)	98.4 (69.5–132.5)**	32.4 (1.5–86.0)	189.0 (85.3–275.0)	188.1 (77.6–610.5)	208.6 (20.3–606.3)	80.4 (18.8–181.2)	< 0.001
Ferritin (μg/ml)	200.0 (185.0–290.0)	242.5 (130.0–520.0)	387.5 (110.0–695.0)**	542.5 (220.0–800.0)	260.0 (130.0–550.0)**	315.0 (220.0–430.0)	< 0.001
Transferrin (ng/ml)	290.0 (270.0–310.0)	202.5 (50.0–380.0)**	353.5 (205.0–630.0)	405.0 (185.0–700.0)	313.8 (205.0–450.0)	154.0 (50.0–402.0)	< 0.001
α 1AT (ng/ml)	205.0 (170.0–278.0)	245.0 (82.5–337.5)	225.0 (99.0–392.5)	251.3 (185.5–368.0)	226.3 (95.0–336.0)	325.0 (227.5–381.0)	< 0.001
α FP (ng/ml)	4.0 (2.0–6.0)	80.0 (7.0–220.0)	23.2 (10.0–152.0)	120.0 (10.0–320.0)	119.6 (54.0–165.0)	240.0 (140.0–396.0)	< 0.001

Values expressed as median (range).
Pair-wise comparison with the post-hoc Dunnett test was done to compare all groups against HCC as a reference group.
* Kruskall Wallis test.
** No significant difference with HCC group.

Table 3 AUC, cut-off values, sensitivity and specificity of different markers for HCC versus control and benign liver diseases.

	HCC vs. control				HCC vs. benign diseases			
	AUC	Cut-off	Sensitivity (%)	Specificity (%)	AUC	Cut-off	Sensitivity (%)	Specificity (%)
TNF-α (pg/ml)	1.000	⩾120.0	100	100	0.992	⩾214.5	100	97.1
EGF (pg/ml)	0.944	⩽100.0	92.5	94.1	0.876	⩾69.0	97.5	89.3
EGFR (fm/ml)	1.000	⩽375.0	100	100	0.903	⩾279.0	97.5	89.3
GST-α (μg/l)	1.000	⩾18.5	100	100	0.956	⩾45.8	92.5	90.4
Iron (μg/ml)	0.775	⩽91.4	77.5	76.5	0.730	⩽90.2	77.5	71.8
Ferritin (μg/ml)	0.983	⩾227.5	97.5	94.1	0.508	⩾305.5	57.5	52.5
Transferrin (ng/ml)	0.974	⩽274.0	97.5	94.1	0.890	⩽232.5	90.0	77.4
α 1AT (ng/ml)	0.995	⩾243.8	97.1	94.1	0.884	⩾279.0	90.0	74.6
α FP (ng/ml)	1.000	⩾73.0	100	100	0.973	⩾158.0	91.4	89.2

AUC = area under the curve.

Table 4 AUC, cut-off values, sensitivity and specificity of different markers of HCC versus HCV and HBV.

Parameters	HCC vs. HCV				HCC vs. HBV			
	AUC	Cut-off	Sensitivity (%)	Specificity (%)	AUC	Cut-off	Sensitivity (%)	Specificity (%)
TNF-α (pg/ml)	1.000	⩾185.0	100	100	0.967	⩾197.5	100	96.7
EGF (pg/ml)	0.975	⩾69.0	97.5	100	0.976	⩾49.0	97.5	100
EGFR (fm/ml)	1.000	⩾267.5	100	100	1.000	⩾250.0	100	100
GST-α (μg/l)	0.843	⩾54.5	80	70	0.987	⩾28.5	100	97.5
Iron (μg/ml)	0.963	⩽116.9	90	97.5	0.954	⩽120.5	90	87.5
Ferritin (μg/ml)	0.954	⩽397.5	90	90	0.703	⩽347.5	67.5	62.5
Transferrin (ng/ml)	0.974	⩽295.0	97.5	92.5	0.970	⩽272.5	97.5	85.0
α 1AT (ng/ml)	0.829	⩾307.5	70	70	0.849	⩾282.8	87.5	80.0
α FP (ng/ml)	0.916	⩾186.0	82.9	82.9	0.997	⩾141.0	97.1	97.2

AUC = area under the curve.

Discussion

Patients enrolled in this study were considered to be at high risk for HCC, namely HBV and HCV infected individuals, schistosomal and cirrhotic patients. Chronic infective hepatitis affects about 3% of the world population. It may lead to persistent hepatocyte necro-inflammation and hepatic fibrosis [17], and is responsible for a large proportion of patients with

Table 5 AUC, cut-off values, sensitivity and specificity of different markers for HCC versus schistosomiasis and cirrhosis.

	HCC vs. Schist				HCC vs. Cirr			
	AUC	Cut-off	Sensitivity (%)	Specificity (%)	AUC	Cut-off	Sensitivity (%)	Specificity (%)
TNF-α (pg/ml)	1.000	⩾131.8	100	100	0.997	⩾232.5	96.7	100
EGF (pg/ml)	0.975	⩾61.0	97.5	100	0.928	⩾64.0	97.5	95.0
EGFR (fm/ml)	1.000	⩾227.5	100	100	0.996	⩾283.8	97.5	100
GST-α (μg/l)	1.000	⩾25.5	100	100	0.976	⩾42.5	95.0	92.5
Iron (μg/ml)	0.893	⩾54.0	87.5	82.5	0.878	⩽54.0	90.0	80.0
Ferritin (μg/ml)	0.810	⩾282.5	80.0	75.0	0.675	⩾292.5	72.5	67.5
Transferrin (ng/ml)	0.625	⩽154.5	52.5	62.5	0.957	⩽246.0	92.5	85.0
A1AT (ng/ml)	0.917	⩾278.8	90.0	85.0	0.893	⩾290.3	85.0	80.0
AFP (ng/ml)	0.978	⩾141.0	97.1	91.4	0.987	⩾158.0	91.4	94.3

AUC = area under the curve.

cirrhosis that ends with HCC and that is mediated by different cytokines [17,18]. The availability of suitable biochemical markers to distinguish between HCC and benign liver lesions would be very useful for early diagnosis. On the basis of the heterogeneity of HCC, the current aim of this study is to discover an accurate and early diagnosis technique for HCC that is based on the simultaneous measurement of a panel of highly specific and sensitive markers, rather than measuring only one. Accordingly, it is necessary to study the role of new biomarkers that might achieve this goal. Different markers with different roles and mechanisms of action that proved to have an impact on the growth of hepatocytes were thus selected. These are pleiotropic inflammatory cytokine (TNF-α), growth factor (EGF and EGFR), antioxidant (GST-α), markers of iron and related proteins (iron, ferretin and transferrin), a protein synthesized in the liver (α1AT), in addition to the classical marker αFP.

The present results indicates that TNF-α has a potential role in diagnosing and distinguishing different liver diseases as its value increases in the following order HCC > HBV > HCV > cirrhosis > schistosomiasis. These results are in agreement with previous studies that revealed elevation of circulating TNF-α level in HCC patients [19]. Byl et al. [20] relate the rise in aspartate transferase to the increased release of TNF-α and IL-1 from Kupffer cells of the liver. It is worth mentioning that Maki et al. [21] report that inflammatory by-products caused by HBV or HCV infection and TNF-α derived from Kupffer cells produce oxygen-derived free radicals and reactive oxygen species. These compounds mediate hepatic fibro genesis and liver injury [21,22]. The TNF-α may also induce the production of other factors that contribute to HCC [18]. Jeng et al. [19] report that TNF-α correlates with disease severity and hepatofibrosis, which may contribute to a high risk for HCC. Sensitivity and specificity of TNF-α for discriminating between benign liver disorders and HCC were 100% and 97.1%, respectively, at a cut-off ⩾ 214.5 pg/ml. Lower levels of TNF-α were highly sensitive and specific when comparing HCC with controls. Accordingly, the present findings recommend TNF-α as a promising tumour marker for HCC. Jeng et al. [19] report that TNF-α correlates with disease severity and hepatofibrosis, which may contribute to a high risk for HCC. In addition, Ataseven et al. [23] found that serum TNF-α significantly increased in cirrhosis and HCC. It is generally believed that increased endogenous TNF-α in advanced liver disease is a consequence of chronic liver failure, which is associated with endotoxin-dependent macrophage stimulation and with a decrease in cytokine clearance [24].

In this study, the serum levels of EGF and its receptor significantly decreased in all the disease groups as compared with the control group. These results coincide with those of Oguey et al. [25] who observed that EGF was down-regulated in rat liver cirrhosis. Burr et al. [26] demonstrated that the decrease in EGFR is associated with decrease of EGF and increase in TGF-α in rodent liver cancer models. However, patients in this study with HCC had significantly higher levels of EGF and EGFR as compared with their corresponding levels in benign liver diseases, but not with normal individuals. In fact, the variation between groups was slight, which makes it difficult to distinguish between malignant and benign liver diseases.

Dash et al. [27] reported that populations at high risk for liver cancer include those who have genetic polymorphism in both microsomal epoxide hydrolase and GST. The levels of these markers may help in identifying susceptible populations in developing countries. All GST isoenzymes are expressed in the liver with different proportions [28]. Many drug-resistance genes such as GST, glycoprotein and heat-shock proteins are expressed by HCC [29]. These data indicate that the determination of GST-α is very useful in the differentiation between HCC and other chronic liver diseases. Previous studies have reported that increased serum GST-α is a sensitive indicator of acute liver damage [30]. Fernandes et al. [31], who observed over expression of GST-α in sera of transgenic mice with chronic hepatitis, report similar findings. In this study, GST-α had 100% sensitivity and 100% specificity for differentiating HCC from normal healthy individuals. These results also indicate high sensitivities and specificities to discriminate between HCC and each of the benign liver diseases that were enrolled in the study.

Because the liver is an iron-rich organ that contains 30% of total body storage [32], it is the most susceptible organ to cellular damage, caused by the abrupt accumulation of iron. In this study, iron ferritin and transferring were slightly elevated in sera of patients with HBV, HCV and cirrhosis, as compared with their corresponding values in sera of normal controls. These data are in agreement with Kowdley [33] who reported that viruses might directly up-regulate hepatic production of the transferrin receptor or ferritin, leading to increased hepatic iron deposition. These increases account for various mechanisms in which iron mediates or modulates response to infection or inflammation. Ferrous iron is a catalyst for free radical formation and lipid peroxidation, which are known to occur in different tissue. El-Atrebi [34] found that there were elevations in serum iron, ferritin and transferrin concentration in patients with the hepatitis C virus. On the other hand, this study revealed lower levels of serum iron and transferrin in both schistosomiasis and HCC groups than in the control group. These results are in agreement with Farag et al. [35]. However, the variation in the levels of iron, ferretin and transferrin obtained in this study are not sufficient to diagnose HCC or other liver diseases. Consequently, the three markers had weak sensitivity and specificity for distinguishing HCC from benign liver diseases.

Similarly, serum alpha-1-antitrypsin did not add much for differentiating HCC from benign liver diseases despite its significantly higher levels compared to chronic liver disease groups. This is obvious from its low sensitivity and specificity. In this respect, it is worth mentioning that El-Asqalani [36] found that α1AT serum levels were significantly increased in HCC and other types of malignant liver tumours as compared with normal controls. Alternatively, a high α1AT value may simply reflect a non-specific acute-phase response associated with tissue injury [37].

More than 70% of HCC patients have high serum levels of αFP. Those with values over 400 ng/ml tend to have greater tumour size, bilobular involvement, massive diffuse type, portal vein thrombosis and lower median survival rate. In spite of that, some reports implicate its limited utility in differentiating HCC from benign hepatic disorders for its high false-negative rates [38], This study revealed a significant increase in αFP serum levels in HCC patients. Other benign liver diseases such as schistosomiasis, hepatitis and cirrhosis also showed elevated levels of the protein, but it was still significantly lower than that of HCC. Silver et al. [39] confirmed that there is a strong relationship between viral hepatitis and αFP production. They also observed that αFP synthesis was related to higher aspartate transferase levels and a lack of uniform destruction and repair that occurs during the hepatitis process or schistosomal infestation. In this study, the αFP at a level of $\geqslant$158.0 ng/ml had a 91.4% sensitivity and 89.2% specificity to distinguish HCC from other benign hepatic lesions. However, several investigators concluded that αFP fails as a reliable marker, mainly because it shows poor sensitivity, ranging from 39% to 65% and a specificity ranging from 76% to 97% [40].

Conclusions

This study found that it is advisable to carry out a battery of markers composed of αFP, TNF-α and GST-α to confirm the diagnosis of HCC and distinguish it from other benign liver diseases, namely HCV, HBV, schistosomiasis and cirrhosis.

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